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FIELD EVIDENCE OF SECONDARY POISONING OF FOXES (*Vulpes vulpes*) AND BUZZARDS (*Buteo buteo*) BY BROMADIOLONE, A 4-YEAR SURVEY

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Abstract

This paper presents the result of a 4 year survey in France (1991-1994) based on the activity of a wildlife disease surveillance network (SAGIR). The purpose of this study was to evaluate the detrimental effects of anticoagulant (Ac) rodenticides in non-target wild animals. Ac poisoning accounted for a very limited number of the identified causes of death (1-3%) in most species. Predators (mainly foxes and buzzards) were potentially exposed to anticoagulant compounds (especially bromadiolone) via contaminated prey in some instances. The liver concentrations of bromadiolone residues were elevated and species-specific diagnostic values were determined. These values were quite similar to those reported in the litterature when secondary anticoagulant poisoning was experimentally assessed. ©1997 Elsevier Science Ltd

Introduction

This study reports anticoagulant (Ac) poisoning in wildlife. The Toxicology Laboratory of the Veterinary school in Lyon is involved in a unique nation-wide network for wildlife diseases surveillance (see material and methods). Ac poisoning is seldom described or investigated in wild animals, despite extensive use of rodenticides in the fields. We observed a series of suspected anticoagulant poisoning in several species and it appeared advisable to evaluate the actual impact of anticoagulant rodenticides on wildlife. A

literature survey also showed that very limited information was available, apart from individual case reports [1, 2, 3, 4].

Ac rodenticides are used in major field-treatments in France during fall and winter. Bromadiolone is used extensively against field vole (*Arvicola terrestris*) and coypu (*Myocastor coypus*) as baits (100 mg/kg or ppm for field uses), either carrots/apples (wet baits) or cereals (dry baits). In this retrospective study, only carrots were distributed. Bromadiolone is only applied by official Pest Control Operators (PCO). Wet baits are buried in holes or by means of a special plough, 15 cm below ground. Field application of bromadiolone is under strict regulatory control [5, 6]. Another Ac compound, chlorophacinone, is widely distributed and used against rats, mice, voles, and other rodents. It is mostly sold as 75 ppm baits (against field-voles) and 50 ppm baits (domestic uses) but also available as a concentrated formula (2.5 g/L). It is less strictly regulated than bromadiolone. Chlorophacinone baits can be prepared by farmers and are usually not buried [5, 6].

Material and Methods

Ac concentration in liver samples was determined with a new High Performance Thin Layer Chromatography (HPTLC) [7]. All reagents were HPLC grade. Briefly, 1 g liver was extracted with acetone (10 mL), centrifugated, filtered, evaporated under a nitrogen flux and resolubilized in 1 mL methanol. 10 µL of the final extract were sprayed automatically with an ATSIII automatic sampler[1] on a 10x20 RP-18 HPTLC plate[2]. The plates were eluted with methanol and orthophosphoric acid (4.72 µM) 9:1, allowed to dry for 20-30 minutes, and read under UV light at 286 nm for spot detection. Each peak recorded was then analyzed by the ScannerII[1] and a solid-phase UV-spectrum was recorded. Samples were compared to standards (8 substances were included, based on the available products in Europe: chlorophacinone, difenacoum, bromadiolone, warfarin, coumachlor, coumatetralyl, difethialone and brodifacoum). Confirmed identification required: Rf identical (± 5%) to one of the standards and UV spectrum comparable, if Rfs' were similar. Results from our laboratory [7], show that there is a very high specificity of this analytical technique (no interfering peaks on blank liver extracts) and high sensitivity (sensitivity defined as % positive results in animals known to be exposed is > 90% in a validation trial). Percent recoveries were also high: around 90% for all compounds tested, with a coefficient of

[1]Camag, Basel, Switzerland
[2]Merck-Clevenol laboratory,

variation below 5%. These results compare favorably with a previously published technique using HPLC procedures [8]. Analyses were conducted on eight Ac compounds. Our validation protocol included the testing of blank liver extracts and of decaying liver extracts (we used buzzard and red fox livers) to determine the specificity of the technique and to be certain that no other endogenic compound could be confused with any of the 8 anticoagulants tested. None of these extracts contained any misleading peak [7].

Ac poisoning was confirmed by: 1) signs and/or lesions compatible with Ac poisoning; 2) liver Ac concentration ≥ 0.2 mg/kg. This value was selected because it is the routine limit of detection of Ac compounds with the analytical technique described above and also because Ac poisoning is always associated with liver concentration well above that value. Routine Ac analysis on hundreds of animals over 10 years in our laboratory never found both clinical evidence of Ac poisoning and Ac liver concentration <0.2 mg/kg [9]. The liver appears as the most reliable organ for confirmation of Ac poisoning. Ac liver concentrations are a cumulative indicator of Ac poisoning because signs develop within 2-10 days after ingesion, i.e. well after all the Ac present in the GI tract has been eliminated.

Samples were submitted to the laboratory according to the SAGIR network procedure. Basically, hunters detect unusual mortality cases of game species in the fields. A SAGIR representative is in charge of the submission of samples of dead animals to the local veterinary diagnostic laboratories. If poisoning is suspected, the appropriate samples are submitted to the ENVL Toxicology laboratory [10, 11].

Ac rodenticides are unique. All the compounds marketed so far have a similar anticoagulant mode of action, manifested by severe hæmorrhages and clotting disorders. It is very characteristic at necropsy, even several days after death. Acs' do not appear to have any subtle subchronic effect on laboratory animals: non specific signs such as anorexia and depression usually precede the clinical signs shortly. Other common hæmorrhagic pathologies in wildlife include trauma (blood will usually clot, at least partially), viral hæmorrhagic diseases (in Lagomorphs especially) and various viral and bacterial diseases. These disorders can usually be distinguished from Ac poisoning at necropsy. When Ac poisoning was confirmed, we tried to obtain information from local authorities regarding the time of treatment in the fields, the compound used, its concentration, the kind of bait used and an estimate of the local field vole population density. Liver Ac concentrations were compared by means of non-parametric statistical tests (Mann-Whitney) , since most data appeared highly skewed to the right. A *p*-value of 0.05 was selected.

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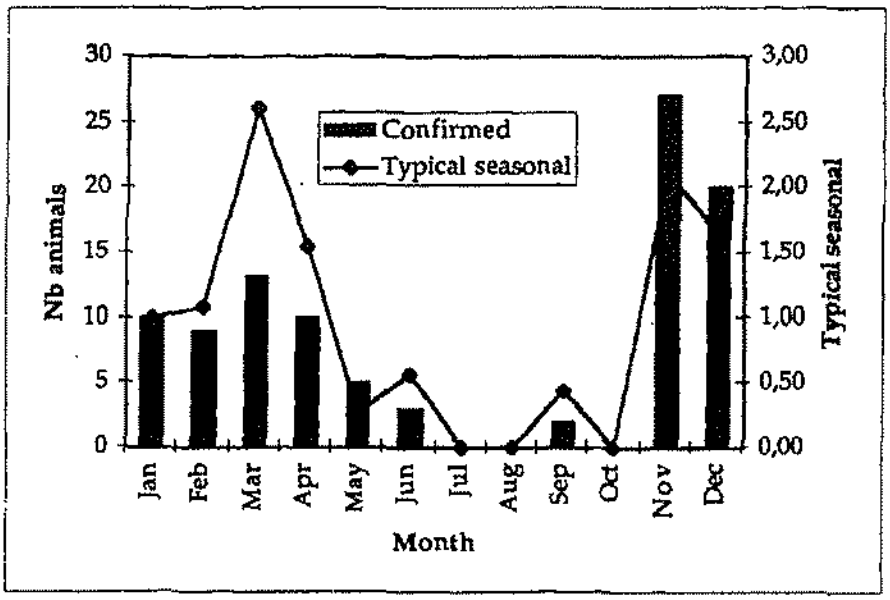
Results

Field and necropsy data

This wide-scale field study includes all the cases received from 1991 to 1994. The number of cases submitted is presented in Table 1. Red foxes (*Vulpes vulpes*) (31 cases), *buzzards (Buteo buteo)* (16 cases) and hare (*Lepus capensis*) (15 cases) were most frequently seen. Many other species were also submitted for suspected anticoagulant poisoning. Table 1 presents data on the number of animals suspected of Ac poisoning, the number of animals submitted for analysis and the number of animals with confirmed Ac poisoning. Most cases occurred during fall and winter (see figure 1). The ratio of Ac poisoning cases to suspected Ac poisoning was maximum in late fall and spring, two major seasons of Ac use in the fields in France (ACTA, 1990). Interestingly, the typical seasonals (indices of the amount of variation attributable to seasonal influences) [12] determined from January 1991 through December 1994, to correct for the annual variations in the number of samples submitted to the laboratory, confirmed this definite seasonal trend, with a peak in late fall and winter-early spring (typical seasonals >1 i.e. statistically significant).

Figure 1: Monthly distribution of animals with Anticoagulant (Ac) poisoning and typical seasonals for Ac poisoning



hæmorrhages, hunters may not consider it necessary to submit samples for analysis (selection bias) and the cost of analysis may be a limiting factor. Regardless, the SAGIR network has been dealing with animals found dead for almost 10 years and investigated thousands of cases which form a very useful databank on wild animal pathology [11].

We found Ac poisoning only occasionnally. Only 188 suspected cases over 5 years, among the several thousands of animals submitted to the network. Ac poisoning is confirmed in less than 1% of the cases submitted to the SAGIR network, especially in the hundreds of animals from game species collected annually. Despite an obvious selection bias, Ac poisoning does not appear to affect the overall populations of wild birds and mammals in France [10, 11], based on the SAGIR samples. The seasonal pattern observed is obviously related to the field use of Ac : primarily in fall and early spring. The seasonal index are maximum in spring. This could be related to the high food intake associated with breeding [9].

Rabbits and hares are very seldom affected (between 1 and 2% of the animals collected each year), although they are likely consumers of treated cereals and carrots. Many animals suffered from viral hæmorrhagic diseases (25-50% of the animals collected) (see table 2) [10, 11]. In non-game species such as foxes and buzzards, Ac poisoning is recognized in a large proportion of cases, but very few animals are submitted each year [10, 11]. Our results confirm a prior report [2] stating that non-target species are not endangered by the appropriate use of Ac rodenticides. They also compare quite well with published data [3] indicating that death attributed to Ac poisoning in barn owls found dead does not account for more than 2% of the animals.

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Fletcher and Grave [4] reported only 6 recent accidents involving rodenticides in Great-Britain. The authors mentioned that birds and mammals found dead after rodenticide use always had direct access to the bait source. Fletcher *et al.* [13] also investigated 763 suspected poisoning incidents in animals in Great Britain in 1993, pesticides were cited as the cause in 212 cases and Ac poisoning in 20 cases (4 cases of brodifacoum poisoning, 8 cases of bromadiolone poisoning and 8 cases of chlorophacinone poisoning in foxes, little owls, mallards, cats and dogs). These accidents were supposedly related to misuse and abuse of Ac.

Among the Ac compounds used only 2 (chlorophacinone and bromadiolone) are of major interest in France. Chlorophacinone was most detected in rabbits and in hares, and in trace

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amounts in the liver of predator species occasionnally. The liver concentrations of chlorophacinone measured in most species are high (usually >1 mg/kg) and comparable to laboratory exposure [15, 16]. The concentrations measured when chlorophacinone was detected in conjunction with bromadiolone were not high compared to cases in which chlorophacinone ocurred alone. When both compounds were detected in an animal, the primary cause of poisoning was probably bromadiolone. Liver bromadiolone concentrations were significantly higher in foxes than buzzards. This is suggestive of higher suceptibility of buzzards to bromadiolone.

Field evidence of poisoning related to the use of bromadiolone is extremely limited. A series of poisoning cases attributed to bromadiolone field-application was reported in Switzerland in 1982 [17], but the bait used was dry and with a higher bromadiolone concentration (140 mg/kg compared with 50 mg/kg in our survey) and residual concentrations in the animals were not published and available for comparison. Furthermore, it was estimated that most of the species involved died of direct ingestion of the bait, because it was a sweet-based product [18].

More striking is the finding that mostly predators (foxes and buzzards) were poisoned with bromadiolone. Direct poisoning of foxes and buzzards after ingestion of a bait, although it cannot be absolutely excluded, appears extremely unlikely for several reasons. Bromadiolone is applied under very strict official control and by PCO's only. It is not likely that foxes and buzzards will eat considerable amounts of carrot or apple-based baits. Wet baits disappear shortly after application (G. Grolleau, personnal communication). If direct bromadiolone poisoning was the most common cause, it should be more common in other species such as rabbit, hare, mallards, etc. and our results show that bromadiolone is seldom detected in these species. Under laboratory conditions, bromadiolone is known to be a potential threat to non-target animals, via secondary poisoning (15). A study was conducted in ermines (*Mustela. hermina*) and buzzards (*Buteo buteo*) [16]. The results indicated that secondary poisoning, although unlikely, was possible in ermines fed bromadiolone-poisoned rodents 5 days in a row. This protocol exceeds what should occur under natural circumstances, since bromadiolone baits are not attractive after 3 days and small carnivores usually do not depend solely on one rodent species for food. Their results also indicated that buzzards could potentially be poisoned by bromadiolone-contaminated rodents after 3 days of consecutive administration or repeated feeding trials (8-10 days apart). Although the number of buzzards affected was limited (2 out of 10), the potential for secondary